The Effectiveness and Safety of commercial HPV vaccines by the Eyedrop route

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Directed by Professor Kyoung Yul Seo

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This certifies that the Master's Thesis of
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ABSTRACT

The Effectiveness and Safety of commercial HPV vaccines by the Eyedrop route

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Mucosal vaccinations can elicit both systemic and mucosal immune responses, but the largest response is the secretion of mucosal secretory IgA (sIgA) antibodies. This response occurs upon entry of most infectious pathogens, such as bacteria and viruses. Mucosal vaccines can be delivered via the eye mucosa, known as conjunctiva, because it is a possible route for mucosal vaccine because it is an important entry site for foreign antigens and pathogens. The conjunctiva have immunological features similar to those of other mucosal tissues.

Human papilloma virus (HPV) causes infections and outbreaks through cutaneous epithelia and ultimately leads to cervical cancer. Cervarix and Gardasil are commercial HPV vaccines that are delivered intramuscularly.

This study showed that delivery of Cervarix by Eyedrop route vaccination induces sufficient antibody production for HPV, especially sIgA, without

mucosal adjuvants, CT or poly(I:C). Additionally, ocular inoculation with Cervarix generated no signs of inflammation within 24 hrs as measured by expression levels of inflammatory cytokine mRNA and the infiltration of mononuclear cells in inoculated sites. There were also no changes in corneal, or retinal morphology or intraocular pressure. Electroretinography showed that the function of photoreceptor cells including rod cells and cone cells, were normal.

Currently, the HPV vaccine is recommended for girls aged 9-13 years by National Immunization Program. Since children feel fear from needles by IM, so there is an advantage to reduce the fear of vaccination by Eyedrop vaccination. Unlike IM, Eyedrop can be easily inoculated without special training.

These results indicated that Cervarix can be a safe and effective mucosal vaccine strategy by Eyedrop route, to induce HPV immunity.

Key words : Human Papillomavirus, Eyedrop vaccination, mucosal immunity

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I. INTRODUCTION

Humans are constantly exposed to environmental irritants, allergens and pathogens which can mount prompt immune responses. Mucosal immunity acts as the first defense barrier because most of these pathogens invade the body through the mucous membrane [1].

Mucosal vaccinations can induce systemic and mucosal immune responses. Mucosal immune system can activate specific mucosal inductive sites with particular effector sites through the concept of 'vaccine compartmentalization', resulting in the generation of secretory IgA (sIgA) to protect against invasion and infection by external pathogens. There are significant degrees of compartmentalization connected specific mucosal inductive sites with particular effector sites because of differentially expressed chemokines, cytokines, and integrins among mucosal tissues. The compartmentalization places constraints on the choice of vaccination route for inducing effective mucosal immune response at the desired sites [2].

Traditional vaccination methods damage the skin, causing pain and increasing the risk of infection, and cause muscle contractions [3, 4]. However, mucosal vaccinations do have either of these disadvantages.

Currently, oral and intranasal vaccines are the most commonly used mucosal vaccines. Intranasal vaccinations stimulate immune responses in nasal-associated lymphoid tissue (NALT), resulting in strong oral, nasal, respiratory, vaginal, and systemic immune responses. Mucosal vaccination can induce strong immune responses with a small amount of vaccines [5, 6].

However, antigens and adjuvants administered through nasal vaccination penetrate into the central nervous system (CNS). This delivery method has been shown to cause facial nerve palsy (bell's palsy) [7]. Unlike intranasal vaccinations, Eyedrop vaccinations were confirmed that vaccine penetration did not occur to the brain even when treated with adjuvant cholera toxin (CT). Influenza vaccines administered through the eyes, have been shown not to cause inflammatory reactions at the inoculation sites [8-10].

The eye mucosa has come to the forefront as a promising vaccination route. Conjunctiva have similar immunological features to other mucosal tissues. Conjunctiva associated-lymphoid tissue (CALT) has $CD4^+$ and $CD8^+$ T cells, mast cells in the lamina propria, dendritic cells (DCs) and Langerhans cells [11, 12]. Therefore, conjunctiva is a possible route for mucosal vaccines.

In our previous study, we reported that Eyedrop vaccination with live-attenuated vaccines, such as influenza virus or Salmonella bacteria, can protect mice from lethal challenge of pathogens [3].

We show that commercial HPV vaccines by Eyedrop route can be an effective and safe measure to induce protective immunity against HPV infection. Gardasil and Cervarix were incorporated into South Korea's National Immunization Initiative Program (NIP) for 12-year-old girls to prevent cervical cancer [13]. Young children can be vaccinated more easily and safely if they are administered by Eyedrop vaccination than by intramuscular (IM) vaccination.

Vaccines are prophylactics, so their effectiveness and safety are important. Safety Vaccinations build strong immune defenses against infections [14].

This study showed that Cervarix vaccine can induce systemic and mucosal

immunization when delivered by Eyedrop vaccination. Cervarix vaccine caused the proper formatting of antibodies and neither caused inflammation at the inoculation sites, or impacted visual functions.

II. MATERIALS AND METHODS

1. Mice

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals promulgated by the Yonsei University College of Medicine.

The committee reviewed and approved this study's animal study protocol (Approval No: 2018-0303). Pathogen-free female BALB/c mice, 6-10 weeks old, were purchased from Charles River Laboratories (Orient Bio, Sungnam, South Korea). The mice were maintained in the experimental animal facility under pathogen-free conditions at the Yonsei College of Medicine (Seoul, Korea) and received sterilized Certified Diet MF food (Oriental Yeast, Osaka, Japan) and filtered tap water ad libitum. All surgeries were performed after sacrificed by CO_2 narcosis and every effort was made to minimize suffering.

2. Vaccine Antigens and Adjuvants

The quadrivalent human papillomavirus (HPV) vaccine Gardasil (Merck, Frankfrut, Germany) and bivalent human papillomavirus (HPV) vaccine Cervarix (GlaxoSmithKline, Brentford, United Kingdom) were used for immunization. Various mucosal adjuvants were used, including cholera toxin (CT) (List Biological Laboratories, Campbell, CA) and polyinosine -polycytidylic acid (poly(I:C)) (Merck, Darmstadt, Germany).

3. Immunization

Before being immunized, the mice were anesthetized by intraperitoneal (i.p.) injection of zoletil (30mg/kg of body weight) and rompun (10mg/kg of body

weight). For IM immunization, 8 μl Cervarix (0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein) and 8 μl Gardasil (0.64 μg HPV-6 L1 protein, 1.28 μg HPV-11 L1 protein, 1.28 μg HPV-16 L1 protein, and 0.64 μg HPV-18 L1 protein) were each suspended in 34 μl phosphate-buffered saline (PBS) and injected at two-week intervals for three times on thigh.

For conjunctival immunization, 8 μl Cervarix (0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein) or 8 μl Gardasil (0.64 μg HPV-6 L1 protein, 1.28 μg HPV-11 L1 protein, 1.28 μg HPV-16 L1 protein, and 0.64 μg HPV-18 L1 protein) with additive 2 μg CT or 10 μg poly(I:C) adjuvants were dropped onto both conjunctival sacs by a micropipette three times at two wk intervals.

4. Sample Collection

At two wks after the third round of immunizations, serums were collected from the mice by retro-orbital bleeding. Tear-wash samples were obtained by lavaging with 10 μl of PBS per eye. Saliva was obtained following i.p. injection with 500 mg/kg of body weight of pilocarpine (Sigma-Aldrich). Vaginal wash samples were obtained by lavaging twice with 50μl of PBS, for a total of 100 μl of PBS per mouse.

5. ELISA for detecting Ag-specific Ab

ELISA plates (Nunc, Roskilde, Denmark) were coated with 8 μl Cervarix / ml PBS (0.14 μg HPV-16 L1 protein and 0.14 μg HPV-18 L1 protein / ml PBS) or 8 μl Gardasil / ml PBS (0.14 μg HPV-6 L1 protein, 0.28 μg HPV-11 L1 protein, 0.28 μg HPV-16 L1 protein, and 0.14 μg HPV-18 L1 protein / ml PBS) and incubated overnight at 4℃. Blocking was conducted with 1% bovine serum albumin (Sigma-Aldrich) in PBS, and two-fold serially diluted

samples were applied to the plates. HRP-conjugated goat anti-mouse IgG or IgA Ab (Southern Biotechnology Associates, Birmingham, AL) was added to each well and incubated overnight at 4℃. Tetramethylbenzidine solution (Thermo Scientific, Rockford, IL) was used for color development. Stopping solution (0.5N HCl) was added to the plates which were measured at wavelength of 450 nm on an ELISA reader (Molecular Devices, Sunnyvale, CA). Endpoint titers of Ag-specific Ab were expressed as reciprocal $\log_2$ titers of the last dilution that showed > 0.1 absorbance over background levels.

6. Histology

The eye specimens, which included the conjunctiva and eyeballs from the control mice and the Cervarix vaccine alone (0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein), the Cervarix vaccine with 10 μg poly(I:C), and the Cervarix vaccine with 2 μg CT treated by Eyedrop vaccinated mice, were washed with PBS and fixed 4% formaldehyde for 24 hr at 4 ℃. The eye tissues were dehydrated by gradual soaking in alcohol and xylene and were then embedded in paraffin. The paraffin-embedded specimens were cut into 5mm thick slices and stained with Hematoxylin & Eosin (H&E).

7. cDNA Synthesis and Real-Time Quantitative PCR

The conjunctival tissue samples were washed with nuclease-free water, and then homogenized at different points in times after Eyedrop immunization with PBS and Cervarix vaccine alone (0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein) or Cervarix vaccine with 10 μg poly(I:C) or Cervarix vaccine with 2 μg CT. Total RNA was extracted using TRIzol (Invitrogen), and cDNA was synthesized with a Maxima First Strand cDNA Synthesis Kit

(Thermo Scientific). cDNA was amplified with Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) and gene-specific forward and reverse primers in an ABI 7300 Real-Time PCR system (Applied Biosystems). Results are expressed as mean ± S.D. after normalizing to the expression of GAPDH gene using the ΔΔCt method.

8. Fundus Photography

The mice were evaluated by fundus photography two wks after receiving their final Eyedrop vaccination. Topical anesthesia was achieved using proparacaine hydroxychloride (Alcaine; Alcon, Fort Worth, TX, USA). Pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine-mixed eye drops (Mydrin-P, Santen Pharmaceutical Co, Ltd.,) before fundus photographs were taken. The fundus photographs were taken with the Micron IV (Phoenix Research Laboratories, Pleasanton, CA, USA), with wavelength range between 450 and 650 nm; the images were stored in Micron IV StreamPix software (NorPix, Inc., Montreal, QC, Canada).

9. Tonometry

The mice were anesthetized by i.p. injection of zoletil (30mg/kg of body weight) and rompun (10mg/kg of body weight). Intraocular pressure (IOP) was measured using a rebound tonometer (Icare® TONOLAB tonometer, Colonial Medical Supply, Franconia, NH, USA).). IOP measurements were made according to the manufacturer's instructions. Six consecutive measurements were taken, and their mean of consecutive trials was used for analyses. Mouse phenotyping analysis was performed by Korea Mouse Phenotyping Center.

10. Optical Coherence Tomography

Mice were anesthetized by i.p. injection of zoletil (30mg/kg of body weight) and rompun (10mg/kg of body weight). The pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine-mixed eye drops (Mydrin-P, Santen Pharmaceutical Co, Ltd.,). OCT scans were conducted using a Micron® IV system (Phoenix Research Labs, Pleasanton, CA, USA). The corneas were lubricated with hypomellose 2.5% (Goniovisc, Hub Pharmaceuticals LCC, Roncho Cucamonga, CA, USA), and the mice were placed laterally in front of the OCT camera on the right side of a platform fixed in front of the OCT lens. Fundus photography and OCT scans and retinal thickness was measured using the InSight-Animal OCT segmentation Software (Phoenix Research Labs, Pleasonton, CA, USA). Each scan of the cornea, lens, and retina were centered on the optic nerve.

11. Elctroretinography

Mice were dark-adapted overnight before the experiment for scotopic testing (to observe the responses of their rod-cell response) and were prepared for recording under dim red illumination. After anesthesia, the pupils were fully dilated using 0.5% tropicamide and 0.5% phenylephrine-mixed eye drops (Mydrin-P, Santen Pharmaceutical Co, Ltd.,). A drop of methylcellulose was placed on the corneal surface to ensure electrical contact and to maintain corneal integrity.

ERG readings were recorded using the Ganzfeld ERG system (Phoenix Research Laboratories) according to the standard protocol given in the instrument's manual. Scotopic ERG readings were obtained with increasing flash intensity from -1.7 to 1.9 log cd/s/m^2. Mice were light-adapted for 15 min prior to the cone-cell response

experiment. Photopic ERGs were conducted with a flash intensity from -0.5 log cd/s/m^2 to 4.1 log cd/s/m^2. 10 times and average response to light stimuli was used in the analyses. The implicit a-wave (as a measure of photoreceptor function), b-wave (as a measure of bipolar cell function), amplitude, and rod and cone cell response were determined.

III. RESULT

1. Significant induction of HPV-specific Ab responses by Eyedrop Cervarix vaccine

This study was conducted to evaluate the effectiveness of commercial HPV vaccines, Cervarix and Gardasil, in inducing systemic and mucosal immunity when administered by Eyedrop route vaccination.

Six wk old female BALB/c mice in the experimental group were inoculated with 0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein in Cervarix by drops in both eyes three times at two wk intervals. To compare with IM immunization, six-week old female BALB/c mice were vaccinated by IM injection above the femur three times at a two wk intervals with 0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein in Cervarix, which were suspended in 34 μl phosphate-buffered saline (PBS). Two wks after final immunization, HPV-specific Abs levels of all mice were measured by ELISA. The experimental group had significantly higher levels of HPV-specific mucosal IgA Abs in mucosal compartments as revealed through tear, saliva, and vaginal washes than compared to mice given PBS or by IM route (Fig. 1A).

The mice in the experimental Gardasil group received 0.64 μg HPV-6 L1 protein, 1.28 μg HPV-11 L1 protein, 1.28 μg HPV-16 L1 protein, and 0.64 μg HPV-18 L1 protein in Gardasil delivered by Eyedrop three times at two wk intervals into both conjunctival sacs by micropipette. For Gardasil IM vaccination, 0.64 μg HPV-6 L1 protein, 1.28 μg HPV-11 L1 protein, 1.28 μg HPV-16 L1 protein, and 0.64 μg HPV-18 L1 protein in Gardasil, each of which was suspended in 34 μl phosphate-buffered saline (PBS) and injected three times at two wk intervals above the femur. Gardasil-specific Abs levels were

measured in serum and various mucosal secretions by ELISA two wks after the final vaccination (Fig. 1B). The Gardasil groups showed slightly lower levels of HPV-specific IgA Abs production in mucosal compartments than the Cervarix groups. The Cervarix group can induce HPV-specific IgA Abs as much as IM route.

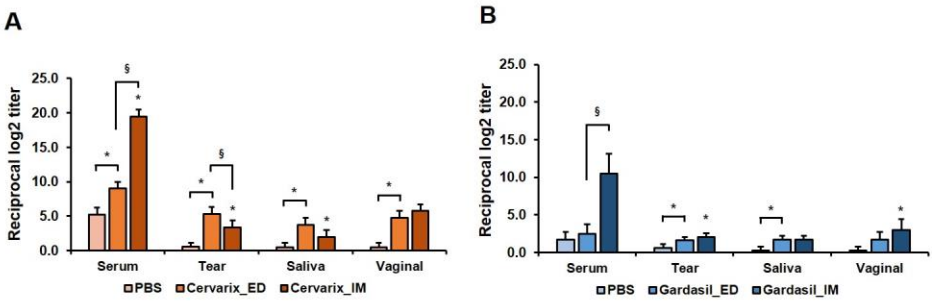

Figure 1. Effectiveness of commercial HPV vaccines by the Eyedrop route. (A) Female BALB/c mice were immunized with Cervarix by drops on both eyes three times at two wk intervals. For IM immunization, Cervarix with phosphate-buffered saline (PBS) was injected three times at two-week intervals above the femur. Cervarix-specific Ab levels were measured in serum and various mucosal secretions by ELISA two weeks after the final vaccination. (B) Female BALB/c mice were immunized with Gardasil and were dropped into both conjunctival sacs by micropipette three times at two wk intervals. For IM injection, Gardasil with PBS was injected three times at two wk interval. Gardasil-specific Ab levels were measured in serum and various mucosal secretions two wks after final vaccination by ELISA. * $p < 0.05$ versus the PBS group; § $p < 0.05$ versus Cervarix or Gardasil alone group. * $p < 0.05$, § $p < 0.05$ compared with control by Wilcoxon matched pairs test. Results are representative of three independent experiments, with four mice in each group.

2. Effectiveness of mucosal adjuvants on commercial HPV vaccines

To evaluate the efficacy of various mucosal adjuvants delivered with commercial HPV vaccines by Eyedrop vaccination, six-week female BALB/c mice were immunized with 0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein in Cervarix or with 2 μg CT or 10 μg poly(I:C) as adjuvants. These mixtures were administered by Eyedrop in both eyes three times at two-week intervals. HPV-specific Abs levels were measured in serum and various mucosal compartments as measured by tear, saliva, and vaginal washes (Fig. 2A). For Gardasil groups, 0.64 μg HPV-6 L1 protein, 1.28 μg HPV-11 L1 protein, 1.28 μg HPV-16 L1 protein, and 0.64 μg HPV-18 L1 protein in Gardasil or Gardasil with 2 μg CT or 10 μg poly(I:C) as adjuvants were dropped into both conjunctival sacs by micropipette three times at two week intervals. Gardasil-specific Ab levels were measured in serum and various mucosal secretions by ELISA two wks after the final vaccination (Fig. 2B).

There were no additive adjuvants effects with Cervarix, but the CT added to the Gardasil had a mucosal adjuvant effect.

The Gardasil with CT group showed higher levels of HPV-specific serum IgG and mucosal IgA levels than the groups which received PBS or Gardasil alone. However, CT administered intranasally, there is a report of CNS damage. Therefore, the following experiments, we focused on assessing the side effects of CT delivered by Eyedrop route.

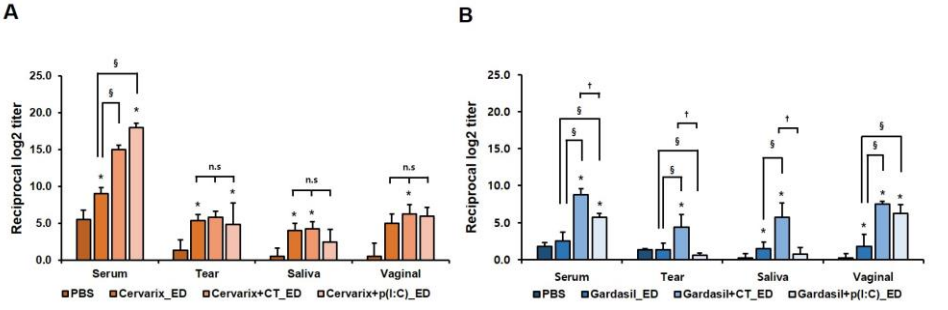

Figure 2. Effectiveness of adjuvants on commercial HPV vaccines. (A) For conjunctival immunization, Cervarix or with CT or poly(I:C) as adjuvants were dropped three times at two wk intervals into both conjunctival sacs by micropipette. Cervarix-specific Abs levels were measured by ELISA in serum and various mucosal secretions two wks after final vaccination. (B) For conjunctival immunization, Gardasil or with CT or poly(I:C) as adjuvants were dropped three times at two wk intervals into both conjunctival sacs by micropipette. Gardasil-specific Ab levels were measured in serum and various mucosal secretions two weeks after final vaccination by ELISA. * $p < 0.05$ versus the PBS group; § $p < 0.05$ versus Cervarix or Gardasil alone groups; † $p < 0.05$ versus Cervarix or Gardasil plus poly(I:C). * $p < 0.05$, § $p < 0.05$, and † $p < 0.05$ compared with control by Wilcoxon matched pairs test. Results are representative of three independent experiments, with four mice in each group.

3. Safety of the administration of Cervarix by Eyedrop route

To identify the safety of Cervarix vaccine by Eyedrop route, we tested to determine whether it could be safely delivered by Eyedrop route without inflammation in the conjunctiva.

Cervarix alone and Cervarix with CT or poly(I:C) as mucosal adjuvants were administered on both eyes. The epithelial and goblet cells from the bulbar conjunctival areas were intact 24 hrs after administration (Fig. 3A). There was no difference in the number of mononuclear cells in sub-epithelial areas in conjunctival tissues of Cervarix alone and Cervarix plus poly(I:C) compared to the PBS group. However, there were more mononuclear cells in tissue of Cervarix plus CT treatment group (Fig. 3B).

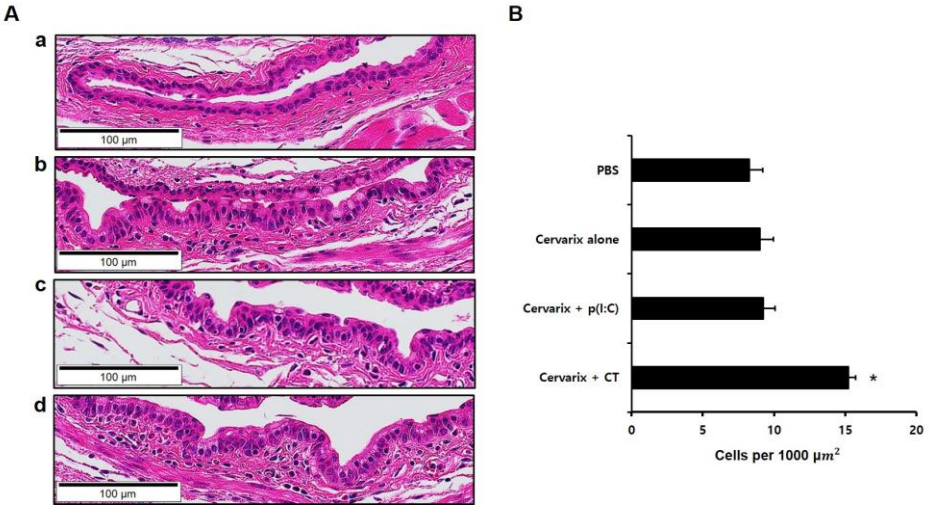

Figure 3. Submucosal inflammation after Eyedrop vaccination. (A) H&E staining of conjunctival tissues. Female BALB/c mice were administered PBS (Aa), 0.64 μg HPV-16 L1 protein, 0.64 μg HPV-18 L1 protein in Cervarix (Ab), or with poly(I:C) (Ac) or CT (Ad) through Eyedrops. (B) Mononuclear cells were counted in the sub-epithelial regions of tarsal conjunctival areas. Results are representative of four independent experiments.

Next, for screening mRNA expression levels of the inflammatory cytokines, RNAs of the conjunctiva were extracted.

Female BALB/c mice were administered 0.64 μg HPV-18 L1 protein in Cervarix alone or with CT or poly(I:C) as adjuvants in both eyes for various time periods for 1hr, 6hr, 12hr, and 24hr. RNAs in the conjunctiva were collected and mRNA of IL-1α, IL-6, TNF-α, and IFN-γ as inflammatory cytokines were quantified by qPCR (Fig. 4).

CT treatment did not induce a significant increase of mRNA expression in the CT treated mice conjunctiva. There are increasing tendencies in CT groups, but they are not statistically significant.

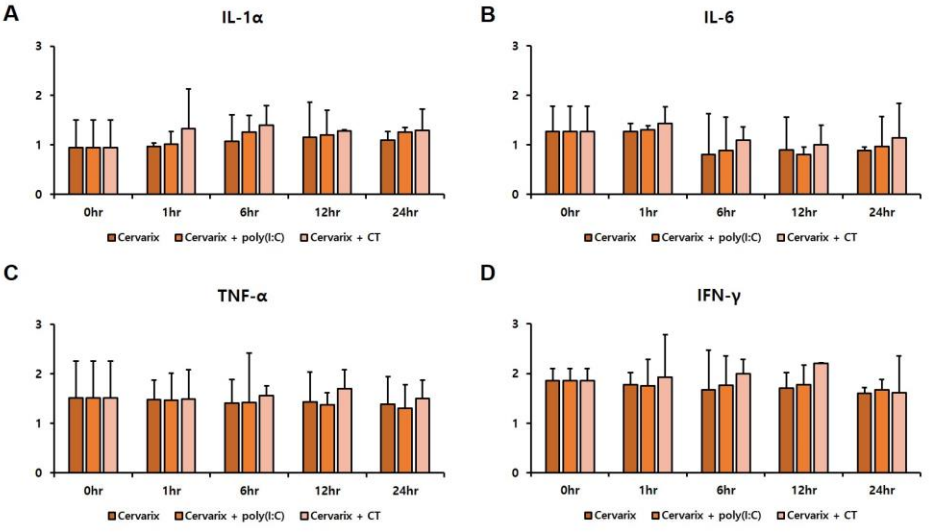

Figure 4. Inflammation induction in the eyes after Eyedrop vaccination. Female BALB/c mice were administered PBS, Cervarix alone or with CT or poly(I:C) as adjuvants in both eyes for various time periods. Total RNA was extracted from homogenized conjunctival tissues with TRIzol reagents for reverse transcription and real-time PCR. Gene expression levels were calculated as the relative ratio to the average value of house-keeping genes, β-actin. Data represent the results of three independent experiments.

The mice were examined to determine whether the Cervarix Eyedrop vaccination treatments provoked external morphological defects as indicators of their safety. A mixture of 0.64 μg HPV-16 L1 protein and 0.64 μg HPV-18 L1 protein in Cervarix alone or with 2 μg CT or 10 μg poly(I:C) as adjuvants was administered in both eyes. There were no changes in the outer eyes or the fundus and there were no cataract lenses (Fig. 5A). The experimental group had similar intraocular pressure as the PBS group (Fig. 5B).

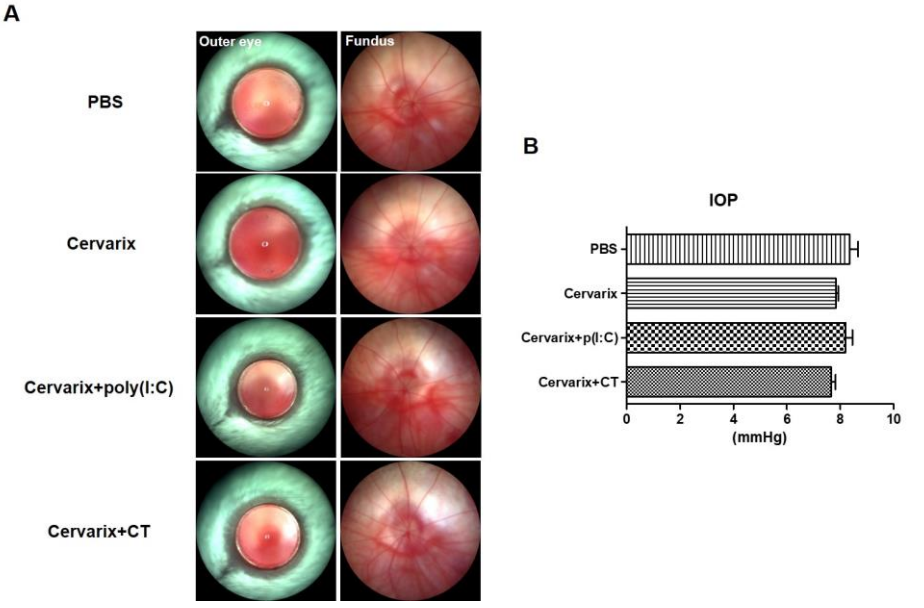

Figure 5. Changes of eye morphology and Intraocular pressure (IOP) after Eyedrop vaccination. (A) The corneal and retinal morphology of the Eyedrop vaccination groups as shown in fundus photographs of the PBS, Cervarix, and Cervarix with adjuvants groups whose eyes showed a radial pattern of arterioles and venules. (B) IOP of the Eyedrop vaccination groups. IOP was measured using tonometry equipment.

The changes of corneal and retinal optical coherence tomography (OCT) readings after Eyedrop vaccination was examined. OCT, showed that the treated mice had normal cornea and retina structure in treated mice.

There were also no differences in observed cornea thickness and retina OCT ration. OCT retinal layer depth analysis showed that there was no statistical significance in the inner plexiform layer (IPL), inner nuclear layer (INL), outer nuclear layer (ONL), or retinal pigment epithelium (RPE) retinal ratios of the treatment groups (Fig. 6B, D).

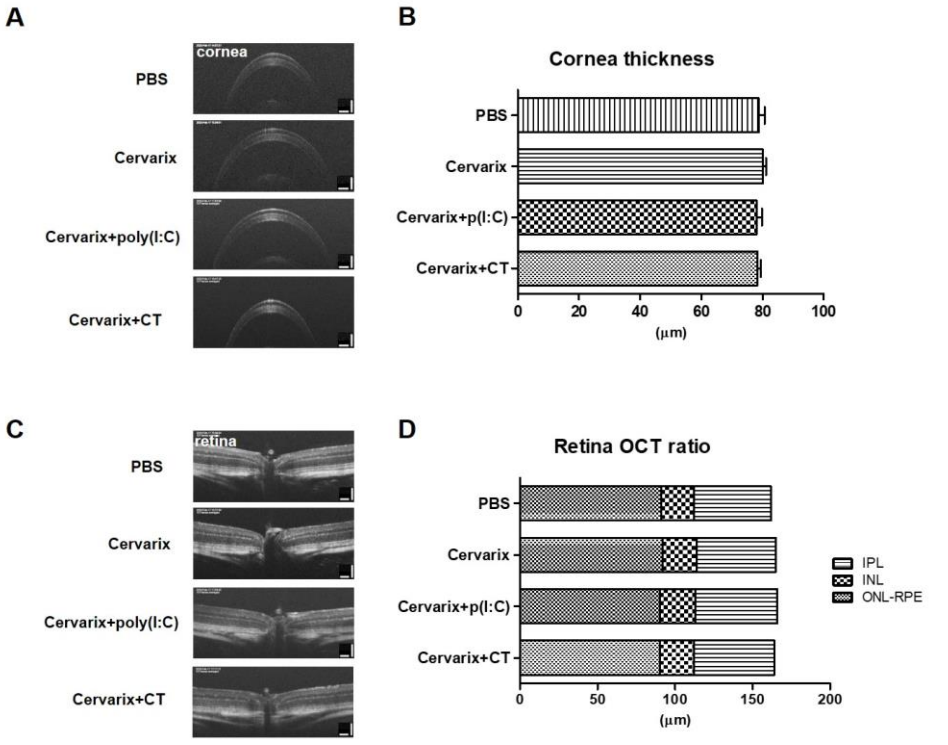

Figure 6. Changes of corneal and retinal optical coherence tomography (OCT) findings after Eyedrop vaccination. (A) Cross-section images of the cornea of the PBS, Cervarix, and Cervarix with adjuvants groups. (B) Cornea thickness of the PBS, Cervarix, Cervarix with adjuvants groups. (C) Cross-section of retina of the PBS, Cervarix, and Cervarix with adjuvants groups. (D) Representative OCT readings, the retinal layer and boundary identifications: inner plexiform layer (IPL), inner nuclear layer (INL), and outer nuclear layer (ONL).

Changes in electrical signals were examined by stage-light stimulation of rod and cone cells. There were no significant differences between the PBS, Cervarix alone, and Cervarix with CT or poly(I:C) groups. (Fig. 7A, B) This result indicates that the Cervarix alone and Cervarix plus CT or poly(I:C) groups had no greater increase in defective morphologies or reduced function in visual acuity than the PBS group.

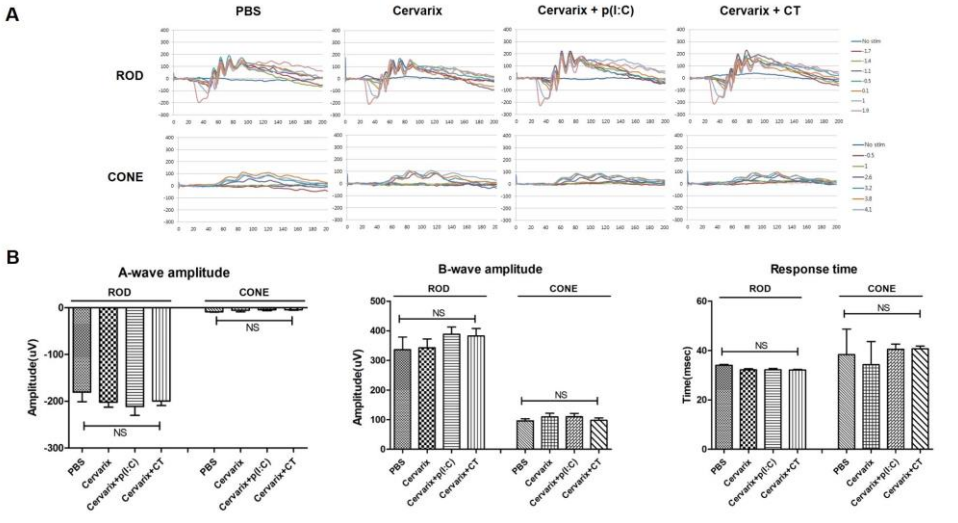

Figure 7. Changes of Electroretinography (ERG) findings after Eyedrop vaccination. (A) ERG of the PBS, Cervarix, Cervarix with adjuvants groups was conducted to determine the light sensitivity of the rod cells, cone cells, and connecting ganglion cells of each mouse. (B) Evaluation of visual function following ERG recording. ERG recordings of photopic and scotopic ERGs were performed on the PBS, Cervarix, and Cervarix with adjuvants groups. NS, not significant.

IV. DISCUSSION

The results of this study showed that Cervarix and Gardasil HPV vaccines induced systemic and mucosal immunization through Eyedrop vaccination. All of the Cervarix groups by Eyedrop route produced more antibodies than Gardasil group. Gardasil group with CT adjuvant had better effects than Gardasil alone group.

The Gardasil with CT adjuvant group was effectively immunized, but inflammation occurred due to mononuclear cellular infiltration at the inoculation sites. However, immunization by Cervarix alone delivered by Eyedrop route induced sufficient immunity against HPV because there were no mucosal adjuvants additive effects. Cervarix vaccine contains an adjuvant, AS04 that consists of aluminum hydroxide and monophosphoryl lipid A (MPL) [15]. It is possible that AS04 already included will affect the immune induction. The effectiveness of MPL as a mucosal adjuvant was investigated oral and intranasal vaccination. It is speculated that MPL as a mucosal adjuvant can also induce sufficient immunity against HPV by Eyedrop vaccination. Unlike Gardasil, Cervarix contains different types of adjuvants that shows saturation for the ability to form antibodies, IgG and IgA. It is likely for this reason that adding mucosal adjuvants did not affect an additive effects in Cervarix vaccine.

When delivered by IM, Cervarix vaccine induced high expression of the inflammatory cytokines, G-CSF, IL-6, KC, and MCP-1 at the injection site [16]. Both IM and Eyedrop vaccinations were administered with the same dose, but Cervarix vaccination by Eyedrop route produced more mucosal IgA antibodies than when delivered by IM.

A large subset of HPV causes infection via cutaneous epithelia. Certain cutaneous HPVs are considered to be high-risk because they cause skin cancers [17]. The role of mucosal IgA is important when considering the path and location

of HPV infection. Therefore, the increased mucosal IgA antibody production caused by Eyedrop vaccination can further reduce the risk of HPV infection.

HPV virus challenge experiments can confirm the immunity to HPV, but no HPV that infects laboratory mice had been identified, until recently [18]. Vaccination with Cervarix alone delivered by Eyedrop route induced sufficient immunity against HPV without causing infiltration of mononuclear cells or disrupting visual function.

Vaccination is a preventative method, so the effectiveness and safety of vaccines is important. The Eyedrop vaccination route is a safe and effective alternative to the intranasal (IN) route. The tear drainage system produces and drains away tears to protect the eyes from harmful environmental particles. The drainage system helps to avoid damage to all parts of the eyes, including the cornea, retina, lens, and optic nerve, caused by foreign bodies [3.] The IN vaccination route causes side effects, such as Bell's palsy. However, Eyedrop vaccinations are not redirected to the CNS in mice [8].

This study showed that the Cervarix vaccine can safely induce sufficient immunization when delivered by Eyedrop route. Cervarix alone can administer on eyes without adding CT or poly(I:C) mucosal adjuvants.

Eyedrop vaccination is easy to administer and does not require special training. HPV vaccination is recommended for routine vaccinations of girls aged 11 and 12 yrs. They are usually afraid of needle injection for IM vaccination, so Eyedrop vaccination is more convenient and easy to vaccinate than IM.

HPV vaccination by Eyedrop route is a safe and effective way to induce immunity, so it can be used as a potent new vaccine strategy for mucosal vaccination.

V. CONCLUSION

This study showed that Cervarix vaccine induced systemic and mucosal immunization through Eyedrop vaccination while Gardasil vaccine did not. The Cervarix group by Eyedrop vaccination did not experience any additive effects due to the CT or poly(I:C) mucosal adjuvants. Therefore, Cervarix alone can induce sufficient immunity when delivered via Eyedrop route.

To examine the possibility of using commercial HPV vaccine by Eyedrop route, we confirmed that delivering Cervarix vaccine by Eyedrop route is an effective and safe alternative to IM injection in mice. Cervarix alone and Cervarix with poly(I:C) and CT mucosal adjuvants delivered by Eyedrop route had the same immunization effects and Cervarix alone was safer for use in the eyes than with adjuvant groups, especially CT.

We found that Cervarix by Eyedrop mice showed a normal retinal phenotype. By means of optical coherence tomography (OCT), we confirmed normal structure of the eye morphology, especially cornea, lens, and retina in both the control and Cervarix by Eyedrop mice. There were no difference in inner plexiform layer (IPL), inner nuclear layer (INL), outer nuclear layer (ONL), or retinal pigment epithelium (RPE) retinal ratio between the two groups according to OCT analysis of the PBS and Cervarix by Eyedrop mice.

Also, they did not change in intraocular pressure (IOP) and electroretinography (ERG). There were no differences between the PBS and Cervarix by Eyedrop mice.

This study demonstrated the effectiveness and safety of delivering the commercial HPV Cervarix vaccine by Eyedrop route in mice. We propose that this will provide new strategies for a potent HPV vaccination route.

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ABSTRACT(IN KOREAN)

점안 접종을 통한 시판 HPV 백신의 효과 및 안정성

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점막 백신 접종을 통해 박테리아와 바이러스 같은 감염성 병원체의 감염에 대항하는 전신 및 점막 면역 반응을 유도할 수 있으며, 특히 점막 lgA 항체를 생성할 수 있다.

안구 점막은 외래 항원 및 병원체의 진입 위치이기 때문에 점막 백신의 경로로 이용될 수 있으며, 안구 점막인 결막 조직은 다른 점막 조직들과 공통된 면역학적 특징을 지닌다.

인유두종 바이러스(HPV)는 피부 상피를 통해 감염 및 발병을 일으키며 자궁경부암을 일으키는 원인이 된다. 이러한 감염 및 발병으로부터 보호받기 위해 시판 HPV 백신인 Cervarix 및 Gardasil을 이용하여 근육 접종을 통해 HPV에 대한 면역력을 획득할 수 있다.

본 연구에서는 점막용 면역보강제인 CT 또는 poly(I:C) 없이 Cervarix 단독으로도 점안 백신 접종을 하였을 때 충분한 면역력을 획득 할 수 있었고, 특히 IgA 항체 생성량이 증가한 것을 확인할 수 있었다.

백신은 예방법 이기에 효과뿐만 아니라 안정성 또한 매우 중요하다.

이러한 점안 접종의 안정성을 확인하기 위해 염증 유발 유무 및 안구 기능을 확인하였다. Cervarix 단독으로 점안 접종 시 염증성 사이토카인의 mRNA 발현이 증가하지 않았으며, 접종된 부위로의 염증성 세포들의 침투가 24시간 내에 일어나지 않았음을 확인했다. 또한 각막, 망막 형태 및 안압의 변화가 정상군과 차이가 없음을 확인하였고, electroretinography (ERG)를 통해 광수용체 세포인 간상세포와 원추세포의 기능도 정상임을 확인하였다.

현재 HPV 백신은 국가예방접종으로 만 9-13세 여아에게 무료로 예방접종을 권하고 있다. 어린 나이의 아이들은 주사바늘로 인한 공포감을 느끼기 때문에 이러한 점안 접종을 활용하여 예방 접종에 대한 공포심을 줄여줄 수 있는 장점이 있다. 또한 점안 접종은 주사 접종과 달리 전문적인 훈련 없이도 손 쉽게 접종할 수 있다.

이러한 결과를 바탕으로, 시판 HPV 백신인 Cervarix의 사용은 HPV에 대한 면역력을 충분히 유도할 수 있으며 점안 접종의 안정성 및 효과를 확인할 수 있었다.

핵심되는 말 : 사람인유두종바이러스, 점안접종, 점막면역